EIF2B5 (IR00002278 / K512 ICS internal reference) mouse line genotyping protocol

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For any question, please contact:

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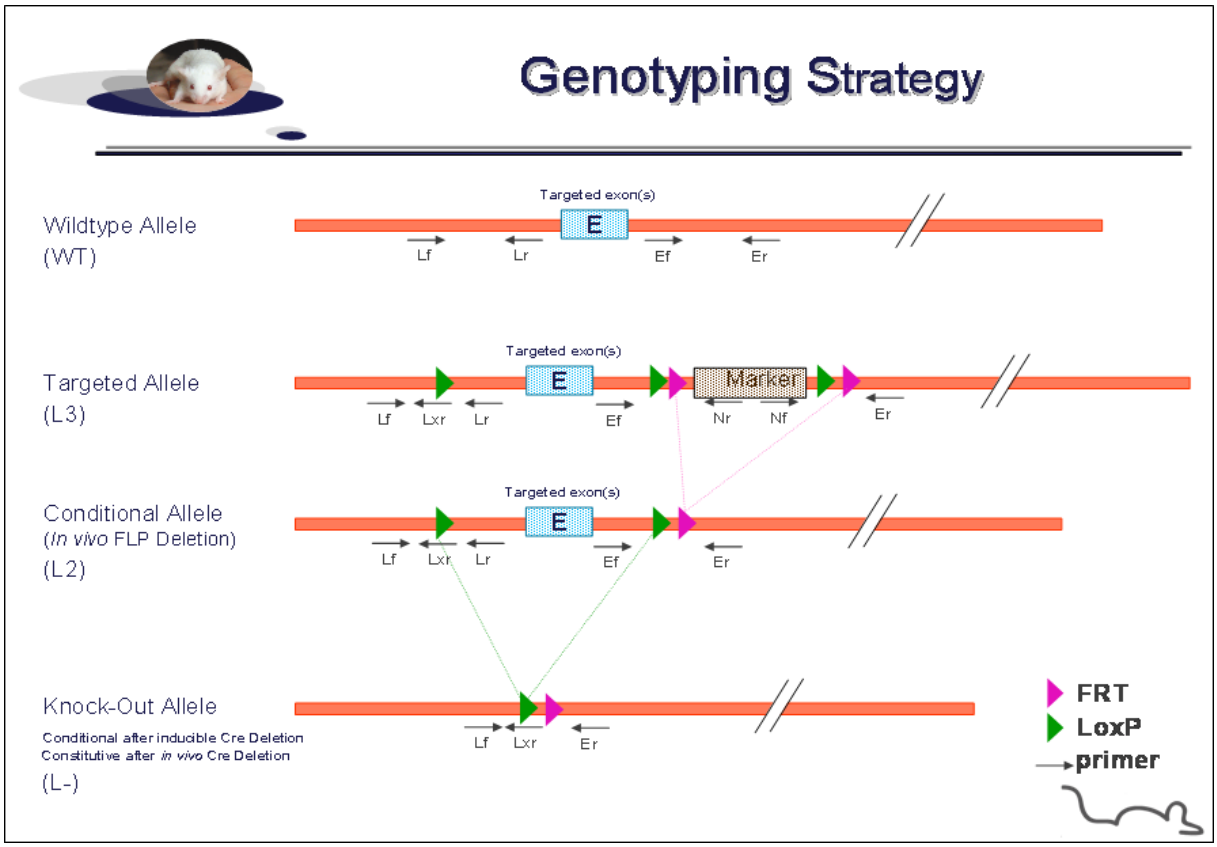
This protocol has been validated by Valérie Rousseau.

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Eif2b5** Conditional Knockout (cKO) project.

1.1. Genotyping strategy

The map below describes the position of the primers used for genotyping for each possible allele.



Sequence of primers used for genotyping

Position	Sequence
Ef	GGAGCCAGTGACCTCTTCTG
Er	TGCTGGGACTGTAAGTGTGG
Er	TTAATCCCAGCACTCGGTCT
Lf	CAACATGTTTTCCATGTTACGG
Lr	ACTCCTCTGTGGCTTTCCTG
Nf	CAGCTCATTCCTCCCACTCATGATC
Nr	TGCTAAAGCGCATGCTCCAGACTGC

PCR fragments expected size (bp):

Region analyzed	Position on the primer (see the map above)	Targeted allele (L3)	cKO allele (L2)	KO allele (L-)	WildType allele (WT)
Presence of the distal loxP	Lf / Lr	382	382	---	289
Excision of the selection marker	Ef / Er	2180*	287	---	179
5' part of the selection marker	Ef / Nr	269	---	---	---
3' part of the selection marker	Nf / Er	377	---	---	---
Excision of the floxed exon(s), i.e. knock out	Lf / Er	4395*	2502*	376**	2301*

* This PCR product will not be observed using our PCR genotyping conditions (see description below)
** This PCR is only verified if mice are generated
--- No Amplicon should be obtained

1.2. PCR protocol

This section describes the composition of the mix and cycling conditions used for genotyping.

Reagents:	Volume:
- FastStart PCR Master (Roche)	7.5µl
- DNA (50ng/µl)	1.5µl
- 5' primer (100 µM)	0.06µl
- 3' primer (100 µM)	0.06µl
- Sterile H_2O	up to 15 µl

Cycling conditions:

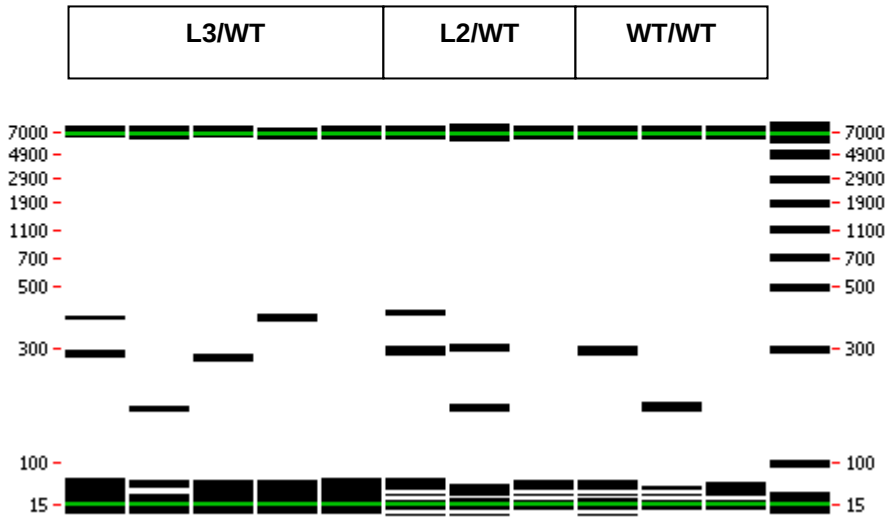
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5 min	1

NB: These PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.

1.3. Picture of genotyping with various alleles

Analysis of PCR products pattern was not done by gel electrophoresis but using LabChip® 90 microfluidic apparatus. PCR products were run on the HT DNA 5K LabChip® 90 Assay Kit.

Representative genotyping picture



Note that as this technology is more sensitive than gel analysis, non specific signals and/or primer dimers may be visible on the picture.